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TITLE: A Fresh Approach to Identification and Characterization of Early Changes of Disease Associated with Ovarian Cancer

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14. ABSTRACT

Our Consortium Development Award allowed us to bring together researchers from MD Anderson, Weizmann Institute, Lawrence Berkley National Labs, Harvard Medical Center, Baylor College of Medicine, Imperial College, Norway Radium Hospital and the University of Toronto in a series of face to face meeting and teleconferences. In this last year, we have consolidated our ovarian data from the various institutions into a centralized database and have added external data only recently available from the TCGA. This has allowed us to develop a strategy on how we can best utilize the talents of our consortium members to address the early molecular changes in ovarian cancer. Based on new data and most importantly input from our consortium members, we will focus our efforts on the early events leading to HGS-OvCa. This is most critical challenge in ovarian cancer as HGS-OvCa constitutes 70% of the cases and 85% from ovarian cancer. Further less is known about the events leading to initiation and progression of HGS-OvCa with even the cell of origin of HGS OvCa.

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INTRODUCTION (Overview and Synergy)

In this consortium, we have taken a bold approach of recruiting a combination of experienced ovarian cancer researchers with a group of thought leaders whose primary expertise is in other diseases and disciplines. This is aimed at bringing a fresh set of concepts supported by the power of emerging technologies, data from the ongoing efforts such as the Cancer Genomics Atlas (TCGA) and the International Genomics Consortium and new systems biology approaches to the understanding of the processes that underlie the development of ovarian cancer. The consortium includes thought leaders across multiple diseases and disciplines from the United States, Canada, Norway, England, Ireland, and Israel. Importantly, the input from all of the members of the consortium over the year of the planning grant resulting in the introduction of new concepts and approaches presented below. Critically, the consortium has arrived at a novel approach that allows us to "time and order" early events using advanced tumors in an unbiased manner.

KEY RESEARCH ACCOMPLISHMENTS
ACHIEVMENTS DURING THE DEVELOPMENT PERIOD

We have accomplished a number of major tasks during our Development Award period. The first was to address the legal and logistical issues that were necessary to proceed to a full Consortium Award. Our Development Award officially started on January 1, 2010; our initial think tank meeting was held January 4, 2010. During our Consortium Development Award, all Research Sites agreed to work collaboratively as part of a partnership to define the early events in HGS-OvCa. We included Natalie Wright from MDACC's Legal Services Department in the Think Tank meeting so that we could address issues pertaining to intellectual and material property coordination. She will retain this role in the full Consortium award. Other administrative details were addressed including coordinating the acquisition of sample material from small OvCA and fallopian tube lesions and setting up sub-groups to assess technology developments able to deal with the limited tissue sample available. The second task was to establish a database that could warehouse data from many different technology platforms. Throughout the year, various members met at research conferences and held multiple teleconferences to work on this task. The database is now rich and useful. However, we will continue

to expand both its information content and utility throughout the Consortium Award. The third task was to develop an expanded repertoire of normal and malignant OvCa lines. This was supported by an ARRA award resulting in the development and characterization of new normal and malignant lines and in particular matched normal and fallopian tube lines from the same patient. The key challenge was to develop approaches that could identify and order the earliest events that occurred during the development of HGS-OvCa. We explored the potential of using small tumors and "early" lesions in the ovary and fallopian tubes, transgenic and knockout animal models and manipulated cell lines. We discarded all of these as key discovery approaches as they have a number of unproven assumptions associated with them. It is not clear that "small or potentially early" lesions in the fallopian tube are indeed early and have not already accumulated a large number of aberrations or further that they are destined to progress and result in morbidity and mortality. The transgenic and knockout animal models and manipulated cell lines all start with an assumption as to what are the earliest events in ovarian cancer, a supposition for which there is not yet adequate support. Further, the transgenic and knockout models as well as the manipulated cell line models may not faithfully recapitulate the underlying process and subsequent genomic events that occur during the early steps leading to the development of human HGS-OvCa. This led to novel approaches to develop a "molecular clock" of aberrations that occurred early in the development of specific HGS-OvCa. This unbiased approach will for the first time let the patient teach us what is important. This also resulted in us refining the composition of our consortium team as indicated above.

REPORTABLE OUTCOMES

STATEMENT OF WORK (From the grant)

MANAGEMENT TEAM (Dr. Mills Lead, Dr. Yarden co-PI, Dr. Stemke-Hale) Timeline: 1 year.

1. Establish communications
    a. Put in place budgets and contracts (started prior to Consortium Start Date)
        i. Establish and implement all contracts and monitoring of compliance and expenditures
        ii. Establish Intellectual Property agreements, Confidentiality agreements and Bi-directional MTAs
    b. Determine a time for bi-weekly management teleconferences
    c. Set up telecommunications link
    d. Establish videoconferencing capabilities
    e. Establish Wiki site
2. First teleconference (month 1)
    a. Determine priorities for the various Teams
    b. Arrange Team teleconferences.
3. Initiate and continue Research Center Site Visits and evaluation
4. Establish EAB to attend Think tank meeting Potential: Jose Baselga, Aron Ciechanover, John Mendelsohn, Director of MDACC SPOREs and PIs of other Consortiums
5. Establish a time for first Think Tank Meeting (within 2 months of start date)
6. Set up Consortium calendar
    a. Collect information on which conferences our team members will attend
    b. Finalize Think Tank Date
7. Set up Think Tank agenda
    a. To defray costs of Think Tank, coordinate MDACC seminars to be given by consortium members.
8. Plan consortium meeting in association with National or International Meeting

We accomplished all of the tasks form the management team. The first Think Tank meeting was held on January 4, 2010. Teleconferences were set up with the sub-groups.

DATA MANAGEMENT AND INTEGRATION TEAM (Dr. Domany Lead, Drs Jurisica, Almeida, Eltonsy) Timeline 9 months

1. Determine what changes are needed to our existing database structure

2. Implement the OVC sDBMS across the consortium
3. Implement the LIMS API across the consortium
4. Identify data mining approaches to identify candidates
5. Systems Approach to organize candidates into pathways and networks

We are still in the process of building our database, but portions are in-place to handle the existing microarray data, mutational data and proteomic data. Our full Consortium proposal relied heavily of analyzing the data from the Cancer Genome Atlas Project (TCGA) and using that data to predict early events in ovarian cancer. Our full proposal explores more data mining approaches and pathway analysis/integration analyses.

TECHNOLOGY EVALUATION TEAM (Dr. Spellman Lead, Dr. Scherer, Dr. Stemke-Hale) Timeline 6 months

1. Compile a list of technologies available across the sites
2. Determine which areas in the proposal require new technologies
3. Investigate new technologies

We reviewed the available technologies and determined that Next Generation exome sequencing followed by validation by more conventional technologies would have the greatest impact in determine the underlying genomic aberrations that drive high-grade serous ovarian cancer.

PROJECT DEVELOPMENT TEAM (Dr. Brugge Lead, Drs. Bast, Børresen-Dale, Calin, Ince, Lin, Liu, Mok, Naora) Timeline 6 months

1. Complete analysis at the DNA, RNA and Protein level across approximately 1000 tumors
2. Recommend potential Aims for the 2010 Consortium Proposal
3. Recommend optimal organization of Research Centers and individuals for the 2010 Consortium Proposal
4. Recommend potential investigators to add or delete for the 2010 Consortium Proposal

Our project development team did suggest changes to our proposed Specific Aims; these changes were driven by the data generated by the TCGA. Our consortium membership was adjusted accordingly. Our new Specific Aims are:

1. **Use a molecular clock to order the development of recurrent mutations and copy number events to identify early lesions in high grade serous epithelial ovarian cancer (HGS OvCa). Team leader: Paul Spellman, Ph.D.** Using preliminary data from the TCGA, we have demonstrated that it is possible to "time" the point at which a chromosome duplicates by quantifying the ratio of mutations present on both duplicated copies of the chromosome to mutations present on only one of the chromosome copies (see Aim 1 for technical details). In practice this will allow us to "time" events that occurred early in tumor development such as mutations, regions of copy number gain and regions of loss of heterozygosity relative to other events such as mutation in p53, somatic mutations in BRCA1/2 and other genes involved in homologous recombination and the consequences of defects in homologous recombination (see project #2). Critically, these studies will be performed with human HGS-OvCa to allow discovery of the earliest events leading to the development of human HGS-OvCa. This will allow us to both time and determine the relative order of events during ovarian tumorigenesis working back to the initial events that led to the initiation of the human tumors. This project provides the timing for the events to be studied in the other aims for their effects on genomic stability as well as on function of ovarian epithelium.
2. **Determine the consequences of loss of Homologous Recombination competence in early HGS-OvCa.**
   Team leaders Shiaw-Yih Lin PhD and Gordon Mills MD PhD Our recently published data as well as the TCGA demonstrates that in addition to germline mutation in BRCA1/2 in HGS-OVCA, there is a high frequency of somatic mutation in BRCA1/2, loss of BRCA1/2 mRNA through promoter methylation and

mutation in other mediators of homologous recombination. Indeed, based on this analysis, approximately 50% of HGS-OvCa have a defect in a process that could contribute to aberrant homologous recombination and sensitivity to synthetic lethality approaches such as PARP inhibitors. However, as defects in homologous recombination are potentially reversible, we with test the hypothesis that an even greater proportion of HGS-OvCa has a defect in homologous recombination at some time during tumor development. Indeed, we have developed a preliminary "fingerprint" that can identify HGS-OvCa tumors that have undergone a defect in homologous recombination at any time during tumor development. We propose to validate this fingerprint and in collaboration with Aim #1 time the defects and consequences of defects in homologous recombination in ovarian cancer. These will be tested in terms of function and therapeutic opportunities in Aim 3 and 4.

3. **Integrate the early events in tumorigenesis in HGS-OvCa into cellular networks and pathways to elucidate functional consequences. Team Leaders: Yossi Yarden, Ph.D., Eytan Domany, Ph.D., and Igor Jurisica, Ph.D.** Genomic events do not occur in isolation. Rather, the events integrate into pathways and networks that alter cellular functions including the oft described "Hallmarks of Cancer". In this aim, the events identified in HGS-OvCa will be integrated into pathways and networks that can elucidate the functional roles of the events in early ovarian cancer. These events will be tested using the unique technologies and platforms available in Aim #4.
4. **Confirm and model the effects of early events in the development HGS-OvCa in human ovarian and fallopian tube cells and HGS-OvCa cell lines to determine their functional and genomic consequences**

   Team Leaders: Katherine Hale Ph.D. Joan Brugge, Ph.D., Tan Ince, M.D. and Joe Gray, Ph.D.

   This aim will function to determine the relevance of the studies from Aim 1-3. We have developed a novel culture approach that allow propagation of normal ovarian and fallopian tube epithelium. Further it allows the development of new ovarian cancer cell lines with a high success rate providing a set of HGS-OvCa that reflect the aberrations in ovarian cancer with higher fidelity.

   Subaim a: To confirm the presence of "early" lesions found in aim 1-3 in small fallopian tube and ovarian cancers

   Using a suite of technologies applicable to the small and formalin fixed paraffin embedded specimens to determine whether the lesions predicted to be early using the approached described above, can be detected in the earliest lesions available to the consortium.

   Subaim b: To model the effects of p53 and BRCA1/2 in concert with early aberrations from Aim 1-3 on genomic instability and cellular function

   As indicated above the major events in ovarian cancer development are genomic instability with defects in p53 and BRCA1/2. Using a novel culture approach validated under an ARRA supplement, we will determine the effects in normal ovarian epithelium and fallopian tube epithelium of altering p53 using dominant negative and shRNA approaches with and without knockdown of BRCA1/2 on genomic instability as described in Aim 1, defects in homologous recombination as described in Aim 2 and on functional outcomes. Our preliminary data already indicates that altering p53 expression in ovarian and fallopian tube epithelium has markedly different functional consequences.

   Subaim b: To systematically alter and drug genomic changes in ovarian cancer

   Based on the order of events identified in aim 1, we will determine the effects of systematic knockdown with siRNA and with drugs targeting components of the amplicons in HGS-OvCa cell lines.

CLINICAL RELEVANCE TEAM (Dr. Gabra Lead, Dr. Hennessy, Ince) Timeline, 2 months

1. This team will interact with the Project Development team to make sure that any Aims of the consortium will have a direct impact on patient management
2. Include information on standard-of-care for ovarian cancer and indicate where current clinical approaches are lacking

Both or clinical relevance team and our advocacy team suggested that we focus only on high grade serous epithelial ovarian cancer. HGS-OvCa is the deadliest of all types of ovarian cancer and the most prevalent; the incidence rate has been steadily increasing in the Western World.

TRAINING AND MENTORSHIP TEAM (Dr. Keyomarsi) Timeline, 1 month

1. Put together a plan that will provide mentorship for young faculty
2. This program will be based on TRIUMPH(Translational Research in Multi-Disciplinary Program)

Dr. Keyomarsi developed the outline for our training and mentorship for young researchers just starting to learn about ovarian cancer, and the Systems Biology department has agreed to fund two post doctorial students if this grant is funded.

ADVOCACY TEAM (Dr. Perlmutter) Timeline, 1 month

1. Develop and invite advocacy team to Think Tank meeting
2. Develop a plan to incorporate advocates in all aspects of the Consortium
3. This program will be based on work that Dr. Perlmutter has done as an advocate for various breast cancer programs

Dr. Perlmutter has worked closely with the management teams and has interacted with the clinical relevance team to keep our research focused on process that will have the greatest impact on patient management.

CONCLUSIONS

This Development grant has allowed us to bring together highly acclaimed researchers both in the field in ovarian cancer and more importantly excellent researchers who have not worked in the field. We feel they will give us a unique perspective and allow us to come up with approaches that have been applied so successfully to other cancer disciplines.